THE ROYAL SOCIETY

High variation in multiple paternity of domestic cats (*Felis catus* L.) in relation to environmental conditions

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Paternity was analysed in two domestic cat (*Felis catus*) populations differing in habitat structure (rural versus urban) and density (234 cats km^{-2} versus 2091 cats km^{-2}). A total of 312 offspring, 76 mothers and 65 putative fathers were typed at nine microsatellite loci in the two populations. Our data showed a high rate of multiple paternity in the urban population (70–83% of litters with more than one father), whereas it was much lower in the rural population (0–22% of litters with more than one), as most males were able to monopolize the entire litter. However, males reproduced as soon as they reached sexual maturity (i.e. ten months of age) in the urban population, whereas most males delayed reproduction to age three years in the rural population.

Keywords: paternity; microsatellites; male reproductive success; domestic cat, *Felis catus*

1. INTRODUCTION

In many polygynous species showing sexual size dimorphism, male reproductive success increases with increasing size (Le Boeuf & Reiter 1988). However, other factors may also influence male reproductive success. In particular, prevailing ecological conditions may lead to different male and female dispersion patterns, which in turn may affect the potential for monopolization of breeding opportunities (Emlen & Oring 1977). The purpose of our study was to assess whether population characteristics such as density and habitat structure can influence the mating system.

The domestic cat, *Felis catus*, provides a pertinent model for studying the effect of ecological conditions on mating behaviour. Among mammals, cats show slight sexual size dimorphism—adult males being on average 20% heavier than adult females (Pontier *et al*. 1995) and having 20% longer canines than females (Pontier & Natoli 1996)—and, interestingly, exist over a wide range of ecological conditions. Human-induced habitat fragmentation and the spatial distribution of human settlements largely determine the density and spatial organization of cat populations in rural areas (Pontier 1993; Pontier *et al*. 1995). Cats in rural populations have owners who feed them and provide shelter in their houses. These cats however may also supplement their diet with natural prey (e.g. small rodents, birds and rabbits), which are generally abundant and widely dispersed across the environment (Liberg *et al*. 2000). In contrast, the stray cats of urban populations are organized around sources of shelter and food (Izawa *et al*. 1982; Calhoon & Haspel 1989), which are provided by 'cat lovers' at a few traditional feeding sites or found at domestic garbage sites. At low density, less than 250 cats km^{-2}, cats are solitary or females can form small groups of closely related individuals (Corbett 1979; Liberg 1981). Each male's territory overlaps that of a female or group of females, but there is little intrasexual overlap (Corbett 1979; Liberg 1981). Urban cats live in large multi-male–multi-female groups reaching very high density (Izawa *et al*. 1982; Corbett 1979; Liberg *et al*. 2000). In these groups, cats exhibit frequent amicable interactions (Macdonald *et al*. 1987) and olfactory recognition of group members versus strangers (Natoli 1985*a*), while females cooperate in rearing offspring (Natoli 1985*b*; Macdonald *et al*. 1987). The social structure of males is organized around a linear dominance hierarchy (Natoli & De Vito 1991). There is general agreement that the cat social system is a recent construct brought about by the changing environment due to human influence (dispersion of resources) (Liberg & Sandell 1988), and by the capacity of domestic cats to be opportunistic (Macdonald 1983).

Although evidence is scant, mating behaviour is also expected to change between low- and high-density populations. In rural areas males seem to be polygynous, fighting aggressively to copulate with as many females as possible (Liberg 1981), whereas in urban populations cats are promiscuous—males and females copulate with several partners (Natoli & De Vito 1991; Yamane *et al*. 1996). Aggressive behaviour among males during the breeding season is not severe, they allow subordinates to remain within the social group and reproduce (Natoli & De Vito 1991).

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2. MATERIAL AND METHODS

(a) *Populations studied*

We compared male reproductive success in two domestic cat populations. One population was situated in an urban setting and the other in a rural environment, both in France, and they were monitored between 1995 and 1998, and between 1991 and 1996, respectively. Demography, spacing and social pattern were also studied. During 1995–1998 the urban population, situated in a hospital district of Lyon (LCR), included 25–28 adult males and 25–38 adult females. This hospital was partly isolated by walls and busy streets. Resident cats of the colony defended the access to the hospital against intruders. Social interactions between members of the population were rarely agonistic and females were observed to cooperate for rearing offspring. Cats were fed on five feeding sites by the hospital staff. Females showed a clear preference for one feeding site, whereas males often visited two or three feeding sites. On average, one male home range overlapped 12 female home ranges (L. Say and D. Pontier, unpublished data). The population density was 2091 ± 291 (s.d.) cats km^{-2} (Fromont 1997). The rural population, Barisey-la-Côte (BAC), is situated in the north-east of France and included 22–28 adult males and 27–39 adult females. Cats were fed mainly by their owners. Housing in the rural environment was clumped within the village on which the population was centred; the nearest neighbouring villages were 3–4 km distant. For the cats, this habitat was fragmented. The density was 234 ± 63 (s.d.) cats km^{-2} (Fromont 1997). Cats lived alone or in small groups (range: two to seven) associated with human dwellings, and were territorial.

(b) *Cat monitoring*

We visually recognized all individuals by their coat colour pattern and fur length or coloured collar. Seventy to 80% of cats were trapped every six months in LCR and once a year in BAC, either by hand or using double-door traps. During each trapping session, cats were anaesthetized with an intramuscular injection of ketamin chlorhydrat (Imalgène 1000 15 mg kg^{-1}, Rhône Mèrieux, Lyon, France) and acepromazin (Vétranquil 5.5% 0.5 mg kg^{-1}, Sanofi, Paris, France). We marked animals using a coloured collar and an electronic device (Transpondeur TROVAN, AEG & TELEFUNKEN Electronic) was injected under the shoulder skin for a permanent individual identification. The age of the cats born in the two populations after 1993 in LCR and after 1991 in BAC was precisely known. These represented, respectively, 84% and 81% of the cats. The age of the other cats was estimated according to Pascal & Castanet's (1978) method. We also collected fur samples from all of the captured animals for genetic parentage analysis.

(c) *Parentage analysis*

We determined paternity for all complete litters we found (i.e. 42 litters corresponding to 192 kittens in LCR, and 31 litters corresponding to 120 kittens in BAC). Genomic DNA samples were isolated using the Chelex protocol: 15–20 hairs were placed in 150 µl of 5% Chelex resin, 15 µl of extraction buffer (pH 8, 0.1 M EDTA, 0.05 M Tris HCl, 1% SDS) and 7 µl of proteinase K (20 mg ml^{-1}), in a 1.5 ml tube and incubated for 4–5 h at 56 °C. Extracts were centrifuged at 12 000 *g* for 2–3 min. The upper layer was taken and mixed with 100 µl of 10% Chelex resin in a 1.5 ml tube and incubated for 20 min at 72 °C.

Selective amplification was carried out for nine microsatellite loci using the polymerase chain reaction (PCR) (25–30 cycles: with 94 °C for 30 s, 55–58 °C for 30 s and 72 °C for 30 s, as denaturing, annealing and extension temperatures, respectively), and the fluorescent-labelled primers fca23, fca43, fca45, fca77, fca78, fca90, fca96, fca8 (Menotti-Raymond & O'Brien 1995), and fca37 (M. A. Menotti-Raymond, personal communication).

(d) *DNA band analysis*

Analysis of the PCR products with 100- and 300-size markers (5 fmol μl^{-1}) were resolved on a 25 ml 6% denaturing polyacrylamide gel on a Pharmacia Sequencer at 45 °C. Data collection and analysis, as well as automatic sizing of bands, was done using Fragment Manager software supplied with the sequencer. Size of bands was obtained using Promega 50–500-external-size marker and Promega 100- and 300-size markers.

The genotype at each of the nine loci was determined for each individual. For each litter, the genotype of each offspring and its mother was used to determine which of the males fathered the offspring through a paternity exclusion procedure. Bands in the DNA profile appearing in the profile of the offspring but not in the profile of its mother were used to assign paternity. The reproductive success of males was estimated annually by the number of kittens they sired.

3. RESULTS

(a) *Structure of the two populations*

The age structure of the adult cat population did not vary significantly among years in either site (LCR: females $\chi^2 = 3.47$, d.f. $= 9$, $p = 0.94$, and males $\chi^2 = 4.44$, d.f. $= 9$, $p = 0.88$; BAC: females $\chi^2 = 12.19$, d.f. $= 15$, $p = 0.66$, and males $\chi^2 = 13.27$, d.f. $= 15$, $p = 0.58$). The two populations did not differ significantly in operational sex ratio, calculated as the proportion of males among the adults (0.49 ± 0.05(s.d.) versus 0.43 ± 0.03(s.d.), $\chi^2 = 0.27$, d.f. $= 1$, $p = 0.60$), or in age structure (Kolmogorov–Smirnov test: $\chi^2 = 0.69$, d.f. $= 2$, $p = 0.71$).

(b) *Paternity study*

We determined the genotype at each of the nine microsatellite loci used for all individuals (65 males, 76 females, 312 kittens) sampled in the two populations. Two different types of litters occurred. We classified a mating as 'one father' when all kittens of a litter had been fathered by a single male, and 'mixed' when at least two different males had sired the kittens of the litter. We observed up to five different fathers for a litter of six kittens (figure 1). The proportion of mixed litters did not vary among years (figure 2). To examine the relationship between the proportion of mixed litters (dependent variable) and the population (independent variable), we applied a logistic regression (using the GLIM package; Francis *et al*. 1993). This proportion was significantly higher in the LCR (mean $\pm$ s.e. $= 76 \pm 2.45$) than in the BAC (12.5 ± 5.19) population ($\chi^2 = 23.66$, d.f. $= 1$, $p < 0.0001$). In the LCR population, non-resident males sired only four kittens out of 192 between 1995 and 1998 (range 0–6% per year). In the BAC population, we found that 19% (range 13–25% per year) of kittens had not been sired by resident males. All non-resident fathers were unable to monopolize females since litters of non-resident males were always mixed litters. The mean age (in years) of males that sired kittens did not differ between the two populations (2.98 ± 1.76(s.d.) in LCR versus 3.89 ± 2.13(s.d.) in BAC; $t = -1.36$, d.f. $= 56$, $p = 0.18$). However, only one cat of

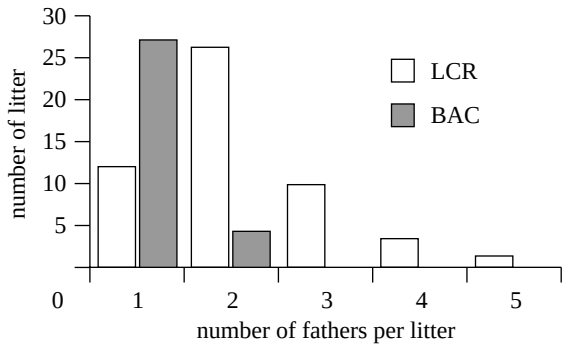

Figure 1. Distribution of the number of fathers per litter in the rural population (BAC) and in the urban population (LCR).

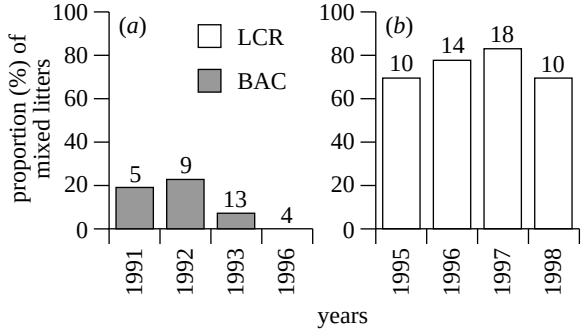

Figure 2. Proportion of mixed litters (at least two different males sired the kittens of the litter) among years in (*a*) the rural population (BAC), and (*b*) the urban population (LCR). The number of litters is given on the graph.

less than three years old sired kittens in the BAC population, whereas two cats as young as ten months old and many 12-month-old cats (20–27% per year) produced kittens every year in the LCR population.

4. DISCUSSION

The study of mammalian mating systems based on behavioural observations has revealed the existence in several species of pronounced variability in mating tactics, both within and between populations of a given species (Lott 1991). However, a molecular genetic approach applied to analyse the variability of male mating tactics such as that described above has been used relatively rarely in mammals compared to birds. As previously suspected, our results confirm that domestic cats are flexible in their mating system (Liberg 1981; Pontier & Natoli 1996) and we think that this variation in reproductive tactics used by males is a response to the constraints set by the environment.

In the BAC (rural) population where females were evenly distributed alone or found in small groups in human dwellings, very few resident males successfully participated in reproduction through monopolization of receptive females. The only male less than three years old that successfully reproduced in this population was of the orange sex-linked genotype. Orange males seem to be more aggressive than males carrying the alternative genotype (Pontier *et al*. 1995, 1998) and aggressivity is an important trait influencing the outcome of male–male conflict (Cox & LeBoeuf 1977). These results are consistent with the existence of a polygynous mating system in males at low population densities. The results also agree with behavioural observations in other rural domestic cat populations of similar density (Liberg 1981; Pontier & Natoli 1996). Liberg (1981) found that a few males were able to control access to receptive females and essentially performed all copulations. He also noted that other males were seen to copulate with the females in the absence of the dominant male. This may explain why some litters were fathered by non-resident males in our BAC study population.

In contrast, in the LCR (urban) population, our molecular results confirm previous data that the cats breed promiscuously (Natoli & De Vito 1991; Yamane 1998). Males successfully mate with several females at each oestrus period and the proportion of litters sired by at least two males was as high as 80%. A tactic consisting of monopolizing the access to a receptive female is presumably not possible due to the strong competitive pressure of many other males (Natoli & De Vito 1991), and thus, females copulate with several males at each oestrus period. Prolonged agonistic interactions among the most competitive males may provide mating opportunities for subordinate males. In this environmental context, males can successfully reproduce as soon as they reach sexual maturity (ten months old), in contrast to the BAC population where males did not successfully reproduce before three years of age.

Considering the scarcity in the literature of studies on paternity determination through molecular analysis in mammals with intraspecific variation in the mating system, this study of the domestic cat provides an important advance in our understanding of mammalian mating systems. We have shown clear differences in the frequency of multiple paternity in relation to ecological conditions and thus provide the first empirical evidence, to our knowledge, for such mammalian mating patterns.

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